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SEQUENCE LISTING

<110> SHYUR, LIE-FEN
CHEN, JUI-LIN
YANG, NING-SUN

<120> A TRUNCATED FORM OF FIBROBACTER SUCCINOGENES 1, 3-1,
4-BETA-D-GLUCANASE WITH IMPROVED ENZYMATIC ACTIVITY AND
THERMO-TOLERANCE

<130> 4910-8

<140> 09/654,652

<141> 2000-09-05

<160> 11

<170> PatentIn Ver. 2.1

<210> 1

<211> 248

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Modified enzyme
with enhanced activity and thermal stability

<400> 1

Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
1 5 10 15

Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala
20 25 30

Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile
35 40 45

Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys
50 55 60

Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala
65 70 75 80

Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln
85 90 95

Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp
100 105 110

Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser
115 120 125

Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu
130 135 140

Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
145 150 155 160

Gln	Phe	Ile	Asn	Trp	Val	Lys	Val	Tyr	Lys	Tyr	Thr	Pro	Gly	Gln	Gly	
				165					170					175		
Glu	Gly	Gly	Ser	Asp	Phe	Thr	Leu	Asp	Trp	Thr	Asp	Asn	Phe	Asp	Thr	
				180					185					190		
Phe	Asp	Gly	Ser	Arg	Trp	Gly	Lys	Gly	Asp	Trp	Thr	Phe	Asp	Gly	Asn	
				195					200					205		
Arg	Val	Asp	Leu	Thr	Asp	Lys	Asn	Ile	Tyr	Ser	Arg	Asp	Gly	Met	Leu	
				210					215					220		
Ile	Leu	Ala	Leu	Thr	Arg	Lys	Gly	Gln	Glu	Ser	Phe	Asn	Gly	Gln	Val	
				225					230					235		
Pro	Arg	Asp	Asp	Glu	Pro	Ala	Pro									
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<210> 2
<211> 267
<212> PRT
<213> Artificial Sequence
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<220>
<223> Description of Artificial Sequence: Modified enzyme
with enhanced activity and thermal stability

<400>	2															
Met	Val	Ser	Ala	Lys	Asp	Phe	Ser	Gly	Ala	Glu	Leu	Tyr	Thr	Leu	Glu	
1				5					10					15		
Glu	Val	Gln	Tyr	Gly	Lys	Phe	Glu	Ala	Arg	Met	Lys	Met	Ala	Ala	Ala	
			20					25					30			
Ser	Gly	Thr	Val	Ser	Ser	Met	Phe	Leu	Tyr	Gln	Asn	Gly	Ser	Glu	Ile	
		35					40					45				
Ala	Asp	Gly	Arg	Pro	Trp	Val	Glu	Val	Asp	Ile	Glu	Val	Leu	Gly	Lys	
	50					55					60					
Asn	Pro	Gly	Ser	Phe	Gln	Ser	Asn	Ile	Ile	Thr	Gly	Lys	Ala	Gly	Ala	
65					70					75					80	
Gln	Lys	Thr	Ser	Glu	Lys	His	His	Ala	Val	Ser	Pro	Ala	Ala	Asp	Gln	
				85					90					95		
Ala	Phe	His	Thr	Tyr	Gly	Leu	Glu	Trp	Thr	Pro	Asn	Tyr	Val	Arg	Trp	
			100					105					110			
Thr	Val	Asp	Gly	Gln	Glu	Val	Arg	Lys	Thr	Glu	Gly	Gly	Gln	Val	Ser	
		115					120					125				
Asn	Leu	Thr	Gly	Thr	Gln	Gly	Leu	Arg	Phe	Asn	Leu	Trp	Ser	Ser	Glu	
	130					135					140					

Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
 145 150 155 160
 Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly
 165 170 175
 Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr
 180 185 190
 Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn
 195 200 205
 Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu
 210 215 220
 Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val
 225 230 235 240
 Pro Arg Asp Asp Glu Pro Ala Pro Asn Ser Ser Ser Val Asp Lys Leu
 245 250 255
 Ala Ala Ala Leu Glu His His His His His His
 260 265

<210> 3
 <211> 349
 <212> PRT
 <213> Fibrobacter succinogenes

<400> 3
 Met Asn Ile Lys Lys Thr Ala Val Lys Ser Ala Leu Ala Val Ala Ala
 1 5 10 15
 Ala Ala Ala Ala Leu Thr Thr Asn Val Ser Ala Lys Asp Phe Ser Gly
 20 25 30
 Ala Glu Leu Tyr Thr Leu Glu Glu Val Gln Tyr Gly Lys Phe Glu Ala
 35 40 45
 Arg Met Lys Met Ala Ala Ala Ser Gly Thr Val Ser Ser Met Phe Leu
 50 55 60
 Tyr Gln Asn Gly Ser Glu Ile Ala Asp Gly Arg Pro Trp Val Glu Val
 65 70 75 80
 Asp Ile Glu Val Leu Gly Lys Asn Pro Gly Ser Phe Gln Ser Asn Ile
 85 90 95
 Ile Thr Gly Lys Ala Gly Ala Gln Lys Thr Ser Glu Lys His His Ala
 100 105 110
 Val Ser Pro Ala Ala Asp Gln Ala Phe His Thr Tyr Gly Leu Glu Trp
 115 120 125
 Thr Pro Asn Tyr Val Arg Trp Thr Val Asp Gly Gln Glu Val Arg Lys
 130 135 140

Thr Glu Gly Gly Gln Val Ser Asn Leu Thr Gly Thr Gln Gly Leu Arg
 145 150 155 160
 Phe Asn Leu Trp Ser Ser Glu Ser Ala Ala Trp Val Gly Gln Phe Asp
 165 170 175
 Glu Ser Lys Leu Pro Leu Phe Gln Phe Ile Asn Trp Val Lys Val Tyr
 180 185 190
 Lys Tyr Thr Pro Gly Gln Gly Glu Gly Gly Ser Asp Phe Thr Leu Asp
 195 200 205
 Trp Thr Asp Asn Phe Asp Thr Phe Asp Gly Ser Arg Trp Gly Lys Gly
 210 215 220
 Asp Trp Thr Phe Asp Gly Asn Arg Val Asp Leu Thr Asp Lys Asn Ile
 225 230 235 240
 Tyr Ser Arg Asp Gly Met Leu Ile Leu Ala Leu Thr Arg Lys Gly Gln
 245 250 255
 Glu Ser Phe Asn Gly Gln Val Pro Arg Asp Asp Glu Pro Ala Pro Gln
 260 265 270
 Ser Ser Ser Ser Ala Pro Ala Ser Ser Ser Ser Val Pro Ala Ser Ser
 275 280 285
 Ser Ser Val Pro Ala Ser Ser Ser Ser Ala Phe Val Pro Pro Ser Ser
 290 295 300
 Ser Ser Ala Thr Asn Ala Ile His Gly Met Arg Thr Thr Pro Ala Val
 305 310 315 320
 Ala Lys Glu His Arg Asn Leu Val Asn Ala Lys Gly Ala Lys Val Asn
 325 330 335
 Pro Asn Gly His Lys Arg Tyr Arg Val Asn Phe Glu His
 340 345

<210> 4
 <211> 744
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: DNA encoding a
 modified enzyme

<220>
 <221> CDS
 <222> (1)..(744)

<400> 4
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 Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
 1 5 10 15

gaa gtt cag tac ggt aag ttt gaa gcc cgt atg aag atg gca gcc gca	96
Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala	
20 25 30	
tcg gga aca gtc agt tcc atg ttc ctc tac cag aat ggt tcc gaa atc	144
Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile	
35 40 45	
gcc gat gga agg ccc tgg gta gaa gtg gat att gaa gtt ctc ggc aag	192
Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys	
50 55 60	
aat ccg ggc agt ttc cag tcc aac atc att acc ggt aag gcc ggc gca	240
Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala	
65 70 75 80	
caa aag act agc gaa aag cac cat gct gtt agc ccc gcc gcc gat cag	288
Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln	
85 90 95	
gct ttc cac acc tac ggt ctc gaa tgg act ccg aat tac gtc cgc tgg	336
Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp	
100 105 110	
act gtt gac ggt cag gaa gtc cgc aag acg gaa ggt ggc cag gtt tcc	384
Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser	
115 120 125	
aac ttg aca ggt aca cag gga ctc cgt ttt aac ctt tgg tcg tct gag	432
Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu	
130 135 140	
agt gcg gct tgg gtt ggc cag ttc gat gaa tca aag ctt ccg ctt ttc	480
Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe	
145 150 155 160	
cag ttc atc aac tgg gtc aag gtt tat aag tat acg ccg ggc cag ggc	528
Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly	
165 170 175	
gaa ggc ggc agc gac ttt acg ctt gac tgg acc gac aat ttt gac acg	576
Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr	
180 185 190	
ttt gat ggc tcc cgc tgg ggc aag ggt gac tgg aca ttt gac ggt aac	624
Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn	
195 200 205	
cgt gtc gac ctc acc gac aag aac atc tac tcc aga gat ggc atg ttg	672
Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu	
210 215 220	
atc ctc gcc ctc acc cgc aaa ggt cag gaa agc ttc aac ggc cag gtt	720
Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val	
225 230 235 240	

ccg aga gat gac gaa cct gct ccg
 Pro Arg Asp Asp Glu Pro Ala Pro
 245

744

<210> 5
 <211> 804
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: DNA encoding a
 modified enzyme

<220>
 <221> CDS
 <222> (1)..(804)

<400> 5
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 Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
 1 5 10 15
 gaa gtt cag tac ggt aag ttt gaa gcc cgt atg aag atg gca gcc gca 96
 Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala
 20 25 30
 tcg gga aca gtc agt tcc atg ttc ctc tac cag aat ggt tcc gaa atc 144
 Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile
 35 40 45
 gcc gat gga agg ccc tgg gta gaa gtg gat att gaa gtt ctc ggc aag 192
 Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys
 50 55 60
 aat ccg ggc agt ttc cag tcc aac atc att acc ggt aag gcc ggc gca 240
 Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala
 65 70 75 80
 caa aag act agc gaa aag cac cat gct gtt agc ccc gcc gcc gat cag 288
 Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln
 85 90 95
 gct ttc cac acc tac ggt ctc gaa tgg act ccg aat tac gtc cgc tgg 336
 Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp
 100 105 110
 act gtt gac ggt cag gaa gtc cgc aag acg gaa ggt ggc cag gtt tcc 384
 Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser
 115 120 125
 aac ttg aca ggt aca cag gga ctc cgt ttt aac ctt tgg tcg tct gag 432
 Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu
 130 135 140
 agt gcg gct tgg gtt ggc cag ttc gat gaa tca aag ctt ccg ctt ttc 480
 Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
 145 150 155 160

cag ttc atc aac tgg gtc aag gtt tat aag tat acg ccg ggc cag ggc	528
Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly	
165 170 175	
gaa ggc ggc agc gac ttt acg ctt gac tgg acc gac aat ttt gac acg	576
Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr	
180 185 190	
ttt gat ggc tcc cgc tgg ggc aag ggt gac tgg aca ttt gac ggt aac	624
Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn	
195 200 205	
cgt gtc gac ctc acc gac aag aac atc tac tcc aga gat ggc atg ttg	672
Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu	
210 215 220	
atc ctc gcc ctc acc cgc aaa ggt cag gaa agc ttc aac ggc cag gtt	720
Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val	
225 230 235 240	
ccg aga gat gac gaa cct gct ccg aat tcg agc tcc gtc gac aag ctt	768
Pro Arg Asp Asp Glu Pro Ala Pro Asn Ser Ser Ser Val Asp Lys Leu	
245 250 255	
gcg gcc gca ctc gag cac cac cac cac cac tga	804
Ala Ala Ala Leu Glu His His His His His His	
260 265	

<210> 6
 <211> 1050
 <212> DNA
 <213> Fibrobacter succinogenes

<220>
 <221> CDS
 <222> (1)..(1047)

<400> 6	
atg aac atc aag aaa act gca gtc aag agc gct ctc gcc gta gca gcc	48
Met Asn Ile Lys Lys Thr Ala Val Lys Ser Ala Leu Ala Val Ala Ala	
1 5 10 15	
gca gca gca gcc ctc acc acc aat gtt agc gca aag gat ttt agc ggt	96
Ala Ala Ala Ala Leu Thr Thr Asn Val Ser Ala Lys Asp Phe Ser Gly	
20 25 30	
gcc gaa ctc tac acg tta gaa gaa gtt cag tac ggt aag ttt gaa gcc	144
Ala Glu Leu Tyr Thr Leu Glu Glu Val Gln Tyr Gly Lys Phe Glu Ala	
35 40 45	
cgt atg aag atg gca gcc gca tcg gga aca gtc agt tcc atg ttc ctc	192
Arg Met Lys Met Ala Ala Ala Ser Gly Thr Val Ser Ser Met Phe Leu	
50 55 60	

tac	cag	aat	ggc	tcc	gaa	atc	gcc	gat	gga	agg	ccc	tgg	gta	gaa	gtg	240
Tyr	Gln	Asn	Gly	Ser	Glu	Ile	Ala	Asp	Gly	Arg	Pro	Trp	Val	Glu	Val	
65					70					75					80	
gat	att	gaa	gtt	ctc	ggc	aag	aat	ccg	ggc	agt	ttc	cag	tcc	aac	atc	288
Asp	Ile	Glu	Val	Leu	Gly	Lys	Asn	Pro	Gly	Ser	Phe	Gln	Ser	Asn	Ile	
				85					90					95		
att	acc	ggc	aag	gcc	ggc	gca	caa	aag	act	agc	gaa	aag	cac	cat	gct	336
Ile	Thr	Gly	Lys	Ala	Gly	Ala	Gln	Lys	Thr	Ser	Glu	Lys	His	His	Ala	
			100					105					110			
gtt	agc	ccc	gcc	gcc	gat	cag	gct	ttc	cac	acc	tac	ggc	ctc	gaa	tgg	384
Val	Ser	Pro	Ala	Ala	Asp	Gln	Ala	Phe	His	Thr	Tyr	Gly	Leu	Glu	Trp	
		115					120					125				
act	ccg	aat	tac	gtc	cgc	tgg	act	gtt	gac	ggc	cag	gaa	gtc	cgc	aag	432
Thr	Pro	Asn	Tyr	Val	Arg	Trp	Thr	Val	Asp	Gly	Gln	Glu	Val	Arg	Lys	
		130				135					140					
acg	gaa	ggc	ggc	cag	gtt	tcc	aac	ttg	aca	ggc	aca	cag	gga	ctc	cgt	480
Thr	Glu	Gly	Gly	Gln	Val	Ser	Asn	Leu	Thr	Gly	Thr	Gln	Gly	Leu	Arg	
145					150					155					160	
ttt	aac	ctt	tgg	tgc	tct	gag	agt	gcg	gct	tgg	gtt	ggc	cag	ttc	gat	528
Phe	Asn	Leu	Trp	Ser	Ser	Glu	Ser	Ala	Ala	Trp	Val	Gly	Gln	Phe	Asp	
				165					170					175		
gaa	tca	aag	ctt	ccg	ctt	ttc	cag	ttc	atc	aac	tgg	gtc	aag	gtt	tat	576
Glu	Ser	Lys	Leu	Pro	Leu	Phe	Gln	Phe	Ile	Asn	Trp	Val	Lys	Val	Tyr	
			180					185					190			
aag	tat	acg	ccg	ggc	cag	ggc	gaa	ggc	ggc	agc	gac	ttt	acg	ctt	gac	624
Lys	Tyr	Thr	Pro	Gly	Gln	Gly	Glu	Gly	Gly	Ser	Asp	Phe	Thr	Leu	Asp	
		195					200					205				
tgg	acc	gac	aat	ttt	gac	acg	ttt	gat	ggc	tcc	cgc	tgg	ggc	aag	ggc	672
Trp	Thr	Asp	Asn	Phe	Asp	Thr	Phe	Asp	Gly	Ser	Arg	Trp	Gly	Lys	Gly	
	210					215					220					
gac	tgg	aca	ttt	gac	ggc	aac	cgt	gtc	gac	ctc	acc	gac	aag	aac	atc	720
Asp	Trp	Thr	Phe	Asp	Gly	Asn	Arg	Val	Asp	Leu	Thr	Asp	Lys	Asn	Ile	
225					230					235					240	
tac	tcc	aga	gat	ggc	atg	ttg	atc	ctc	gcc	ctc	acc	cgc	aaa	ggc	cag	768
Tyr	Ser	Arg	Asp	Gly	Met	Leu	Ile	Leu	Ala	Leu	Thr	Arg	Lys	Gly	Gln	
				245					250					255		
gaa	agc	ttc	aac	ggc	cag	gtt	ccg	aga	gat	gac	gaa	cct	gct	ccg	caa	816
Glu	Ser	Phe	Asn	Gly	Gln	Val	Pro	Arg	Asp	Asp	Glu	Pro	Ala	Pro	Gln	
			260					265					270			
tct	tct	agc	agc	gct	ccg	gca	tct	tct	agc	agt	gtt	ccg	gca	agc	tcc	864
Ser	Ser	Ser	Ser	Ala	Pro	Ala	Ser	Ser	Ser	Ser	Val	Pro	Ala	Ser	Ser	
		275					280					285				

tct agc gtc cct gcc tcc tcg agc agc gca ttt gtt ccg ccg agc tcc	912
Ser Ser Val Pro Ala Ser Ser Ser Ser Ala Phe Val Pro Pro Ser Ser	
290 295 300	

tcg agc gcc aca aac gca atc cac gga atg cgc aca act ccg gca gtt	960
Ser Ser Ala Thr Asn Ala Ile His Gly Met Arg Thr Thr Pro Ala Val	
305 310 315 320	

gca aag gaa cac cgc aat ctc gtg aac gcc aag ggt gcc aag gtg aac	1008
Ala Lys Glu His Arg Asn Leu Val Asn Ala Lys Gly Ala Lys Val Asn	
325 330 335	

ccg aat ggc cac aag cgt tat cgc gtg aac ttt gaa cac taa	1050
Pro Asn Gly His Lys Arg Tyr Arg Val Asn Phe Glu His	
340 345	

<210> 7
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<400> 7	
tcaccacat ggtagcgca aag	23

<210> 8
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<400> 8	
gccacgaatt ctgttcaaag ttcac	25

<210> 9
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<400> 9	
cagccggcga tggccatggt tagcgca	27

<210> 10
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 10

ctgctagaag aattcggagc aggttcgtc

29

<210> 11

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
illustrative peptide

<220>

<221> MOD_RES

<222> (2)

<223> An uncharged residue, such as Alanine, Proline or
Glutamine

<400> 11

Pro Xaa Ser Ser Ser Ser

1

5